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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ^{6} : **G01T 1/29, 1/203**	**A2**	**(11) International Publication Number: WO 96/19739** **(43) International Publication Date: 27 June 1996 (27.06.96)**

(21) International Application Number: PCT/EP95/05132

(22) International Filing Date: 22 December 1995 (22.12.95)

(30) Priority Data:
9425893.6 22 December 1994 (22.12.94) GB

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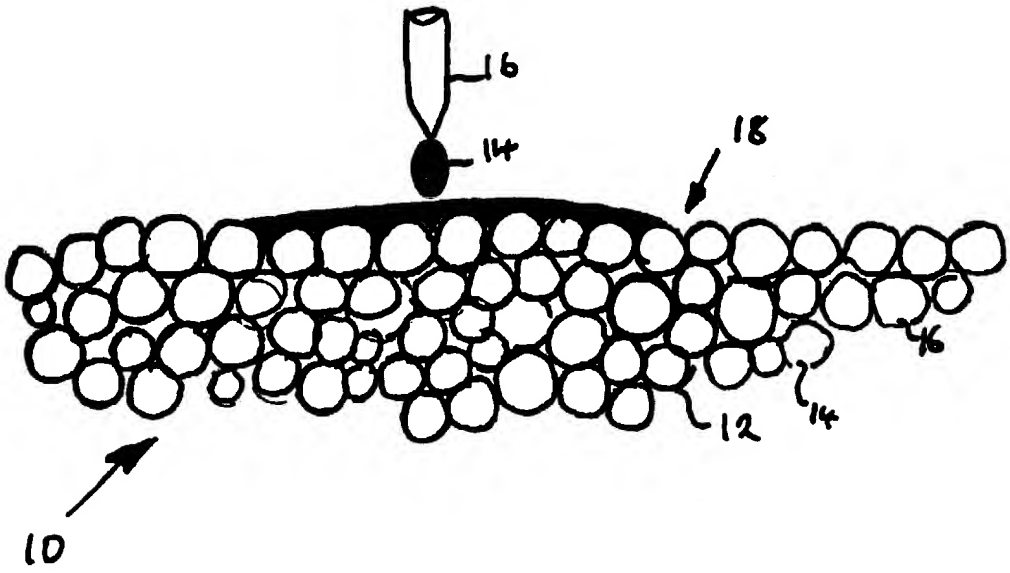
(81) Designated States: AU, CA, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: ASSAY APPARATUS



(57) Abstract

The invention provides a solid support for example for use in a radioligand binding assay, the solid support comprising a plurality of interconnected elements arranged to provide interstitial spaces in which a liquid can flow. When a liquid sample is applied to a surface of the support of the invention it spreads across the surface of the support to a certain extent and also flows into the support on the surfaces of the interconnected elements which form the support and into the interstitial spaces therebetween. The support of the invention may be used as a support for a chemical reaction or for cell growth.

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ASSAY APPARATUS

This invention relates to a solid support which may, in particular, be used as or for an assay apparatus and is particularly, though not exclusively concerned with an assay apparatus for high throughput screening ("HTS") of compounds for pharmaceutical or other properties. The invention also relates to the use of the support as a support for cell growth.

HTS requires automated systems for testing compounds to be screened. Plastic plates containing scintillators have been developed for HTS in which the scintillator is triggered by a radiolabel, such as ^{3}H. The only radioactivity detected is that bound to a specific binder attached to the plastic surface. Such plates are described in International Patent application no. PCT/FI89/00191.

There has been significant interest recently in the development of so called "free format" assay apparatus which is able to analyze samples presented in different formats, for example, in plates with different numbers of wells.

According to one aspect of the invention there is provided a solid support for example for use in a radioimmunassay, the solid support comprising a plurality of interconnected elements arranged to provide interstitial spaces in which a liquid can flow. When a liquid sample is applied to a surface of the support of the invention, it spreads across the surface of the support to a certain extent and also flows into the support on the surfaces of the interconnected elements which form the support and into the interstitial spaces therebetween. Preferably, the size of the interconnected elements, and therefore the size of the interstitial spaces, is controlled so that the applied liquid sample is held within the support by surface tension. The size of the interstitial spaces may also be controlled by controlling the packing of the elements.

The solid support of the invention has various advantages. For example, the solid support of the invention can be formed into a variety of suitable shapes. In particular, the support may conveniently be provided in the form of a sheet which can be readily cut to size prior to use. This makes the support particularly suitable for use in automated screening systems for HTS, and especially systems using a free format analyzer.

The elements which form the solid support of the invention may be porous.

Preferably, the solid support of the invention comprises plastic beads, which may be hollow or porous, sintered or otherwise fused together to form a solid in which the interstitial spaces between the beads can be occupied by a liquid sample applied to a surface of the support. Sintering of the plastic beads may be achieved by temperatures above 200°C together with high pressure or by pressure alone.

Typically, the beads will have a diameter in the micrometer to millimetre range.

Where the solid support of the invention is formed from a plastic material, the beads may be made of a plastic material such as polystyrene.

Preferably, the solid support of the invention is formed from a plastic scintillation material, that is to say a plastic material including a fluorophore such as diphenyloxazole (PPO) or 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP),whereby the material will scintillate in the presence of a suitable radioactive material. Preferably, the radioactive material is a radiolabel present in a sample applied to the support of the invention.

Where the support of the invention is used as a scintillation plate, it may be formed from a material which is translucent, more preferably transparent.

According to another aspect of the invention there is provided an assay method, the method comprising applying at least one sample to be analyzed to a surface of support in accordance with the invention and assaying the or each sample for the presence or absence of an analyte in the sample and/or for a quantifiable or qualitative effect.

The sample may be analyzed several times for different analytes and/or for different quantifiable/qualitative effects, but with only the liquid handling steps for one assay. For example, binding of one hormone labelled with ^{125}I to its receptor and binding of another hormone labelled with ^{3}H to its receptor in one sample can be measured as ^{125}I and ^{3}H have different radioactive energies.

The support of the invention may be used as a support for a chemical reaction. For example, the support may be used as a support for DNA or polypeptide synthesis.

The solid support of the invention may be used advantageously for cell growth. In particular the solid support may be used with certain types of cells which will only express certain proteins when grown in three dimensions as opposed to growth in a two dimensional monolayer. For example, mammary tumour cells will only express lactyl albumin when grown in three dimensions (which is thought to mimic *in vivo* conditions). Thus according to a further aspect of the invention there is provided a growth support comprising a multiplicity of interconnected substantially spherical elements arranged to provide a multiplicity of interstitial spaces therebetween in which cells can grow in, between and/or on the spherical elements. The growth support is advantageous in that a greater amount of cells can be grown in a given unit of surface area compared to two dimensional growth plates. Therefore more growth products can be obtained.

According to another aspect of the invention there is provided a method of growing cells, the method comprising growing the cells in the interstitial spaces between the spherical elements or another surface of the growth support of the invention.

This is advantageous because it permits the reduction of the reaction volume required. For example, in the experiments below, reaction volumes were reduced from 1μl to 30 μl by placing a disc of the support in the wells of a conventional 24 well assay plate.

Whilst there has been a trend towards miniaturization of assay systems for HTS - to 384 and even 864 well plates from 96 well plates or tubes - the instrumentation used for measuring the changes has not kept pace with increasing number of wells tested. In order

to reduce assay volume so as to minimize expenditure on reactant and test compounds Sephadex or Sepharose gel beads have been placed in wells or in larger diameter dishes such as Petri dishes - "gel permeation" - used for example in melanophore assays.

The production of a support in accordance with the invention and its uses will now be described, by way of example only, with reference to the accompanying drawings, Figs 1 to 4 in which:

Fig 1 is an enlarged cross section through a support in accordance with the invention;

Fig 2 is shows the pattern of analyte spread out on the surface of the support of Fig 1; and

Fig. 3 illustrates a binding competition experiment using the support of the present invention; and

Fig. 4 illustrates a dissociation rate experiment using the support of Fig. 3.

MANUFACTURE OF THE PLATE

The support 10 shown in Fig 1, which comprises a plate 12, was formed by mixing Dowex 1-resin (Sigma 1x2-200, dry mesh 100-200, chloride form strongly basic anion exchange) polystyrene beads, e.g. 14, 16 together with a polystyrene solution. The polystyrene solution was produced by dissolving either a conventional polystyrene microtitration plate, a Greiner plate, or ScintiStrips(trade mark) (Wallac) in a solvent (ethyl acetate). Each polystyrene bead is about 100 μm in diameter.

Approximately, 30ml of the resin beads were mixed with 5 ml. of the polystyrene to form a suspension. The suspension was mixed with a stainless steel spoon and spread out on a sheet of aluminium foil as an even layer approximately 2 mm thick. The layer was dried at about 70°C on a thermostated hot plate to form a spongy plate. The aluminum foil was then carefully removed. The side of the plate which had faced the aluminum foil was relatively impervious, whereas the porous nature of the other major face of the plate was clearly apparent.

A plate formed using dissolved ScintiStrips was highly fluorescent in UV light whereas a plate formed using dissolved Grenier plates was not as fluorescent under the same conditions.

When a drop of liquid sample is applied to the surface 18 of the support 10, it spreads across the surfaces shown in Fig 2, which shows coloured saline spots applied to the surface of the plate and also enters the interstitial spaces between the sintered beads. After a number of samples have been applied to the plate, it can be used with a suitable free format scintillation counter to determine the amount of radioactive label in the sample. The sample is localized within the support by surface tension, avoiding the need for a plate containing discrete wells. This facilitates the use of the plate in automated screening systems for HTS.

RECEPTOR BINDING EXPERIMENTS

A fluorescent plate in accordance with the invention was manufactured as described above. using dissolved ScintiStrips, and then cut in half.

In order to confirm that the test specificity of [3H]-estradiol was binding to the receptor rather than the plastic support and to test competition and binding kinetics, a support in the form of a sheet was made as described above. Human estrogen receptor hormone binding domain (hER-HBD) in yeast extracts was diluted 1:100 in regular phosphate buffered saline (PBS). A SPLAT sheet was incubated with the receptor solution without any washing steps of the sheet prior to receptor incubation. A non-programmed yeast extract and plain PBS were used as controls. The sheets were incubated for a total of four days to allow adsorption of protein.

On the day of the binding experiments, the sheets were not washed but simply punched to produce tablets (3 mm in diameter and 1.5 mm thick). The tablets were put into wells of a 24-well dish.

Binding experiment 1:

A binding competition curve was constructed where 30 μl of reaction solution was put onto

each tablet. The tablets were divided into three groups: 1) hER-HBD, 2) non-prog. yeast and 3) plain buffer. The same series of samples were used with all three groups. The samples consisted of PBS with one concentration of radioligand ([3-H]-estradiol; 5 mM) and one given concentration of diethylstilbestrol (DES) in a series of concentrations (0.7, 7, 70, 700 and 7000 nM and no DES). After putting on the sample with a Finnpipette, the tablets were incubated at room temperature for 2 hours. The sheets were measured in a Wallac Microbeta. Data was analysed using Excel (Microsoft, USA). It is significant to note that there was no need to wash the tablets prior to analysis, and plotted using Kaleidagraph.

Figure 4 shows the results of the equilibrium binding competition experiment. There was no binding of [3H]-estradiol to tablets that were incubated with non-programmed yeast extract or with plain PBS. There was, as expected, binding of [3H]-estradiol to sheets treated with hER-HBD. With the latter there was also a concentration dependent inhibition of binding of [3H]-estradiol by DES.

Binding experiment 2:

hER-HBD tablets that had maximal binding-levels in experiment 1, above, were put into the position in a 24-well plate for counting of dissociation rates. To the tablets were added 100 µl of the [3H]-estradiol-only solution used above (vehicle) or the vehicle plus a 3000x excess of DES. Each tablet was now floating in solution. In the experiment 1, above, all solution was absorbed. The scintillation of the tablets was counted repeatedly once every 15 seconds for approximately 30 minutes in a Wallac Microbeta. Data was handled using Excel and plotted using Kaleidagraph.

[3H]-estradiol bound to the receptors declined bi-exponentially as previously described (Häggblad J, Carlsson B & Raynaud J-P (1994) Evaluation of conformational changes in hER-HBD by pharmacological dissection of hormone dissociation rates in a homogeneous hormone binding assay. In : Proceedings; Hormonal Carcinogenesis. Springer Verlag (in press)) in the DES containing solution (Figure 4). With the solution containing [3H]-estradiol only, there was association of [3H]-estradiol to the receptors.

The first result is a typical dissociation curve. The second result is more complex and probably reflects a binding of radioligland to as yet unoccupied sites on the tablets. The latter result suggests that there is an excess of receptors over radioligland and that a new state can be reached by the addition of [3H]-estradiol.

The above results confirm binding of [3H]-estradiol using the support of the invention is to hER-HBD and not to the plastic or to yeast proteins; i.e. [3H]-estradiol binding is specific to receptors. Furthermore, it has been demonstrated that the format of the present invention works in both equilibrium and in kinetic experimental situations, and that it is a very robust format since no washing steps were required during this set of experiments.

Figure legends:

Figure 1. Binding competition curve in the SPLAT format. SPLAT tablets were pretreated with hER-HBD (yeast) or with yeast extract or simple PBS. There is only binding of [3h]-estradiol to hER-HBD pre-treated tablets. The binding of [3H]-estradiol is inhibited by diethylstilbestrol.

Figure 2. Dissociation rate experiment with [3H]-estradiol liganded hER-HBD pre-treated SPLAT tablets. Diethylstilbestrol induces dissociation of [3H]-estradiol. Addition of [3H]-estradiol only, causes association which is explained by an excess of unoccupied receptors to radioligland ([3H]-estradiol).

Whilst in the above example radioactivity was measured using discrete discs cut from a plate of the present invention, it will be appreciated that preferably an entire plate may be analysed using a free format counter such as an image-enhanced CCD camera.

In an alternative embodiment, the beads which form the basis for the support may contain a fluorophore such as diphenyloxazole (PPO) or 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP), which will scintillate in the presence of a suitable radioactive material. Typically, the radioactive material is a radiolabel present in a liquid sample applied by a pipette to the support.

The surface of the plate may be treated chemically or physically by known means to enhance the binding of cells or other molecules to the surface.

The solid support described above may be used as a growth support for cells preferably if it is made from beads lacking a flurophore.

A rigid support in accordance with the invention may also be provided in which the interstital spaces between the elements are in the form of capillaries.

CLAIMS

1. A solid assay support comprising a plurality of interconnected elements arranged to provide interstitial spaces in which a liquid can flow.

2. A solid assay supporting according to claim 1 in which the elements are substantially spherical.

3. A solid assay support according to claim 1 or 2 in which the size of the interconnected elements and therefore the size of the interstitial spaces is controlled whereby an applied liquid sample is held on or within the support by surface tension.

4. A solid assay support according to claim 1, 2 or 3 is in the form of a sheet.

5. A solid assay support according to claim 4 which can be readily cut to size prior to use.

6. A solid assay support according to any preceding claim in which the elements which form the support are porous.

7. A solid assay support according to any preceding claim in which the interconnected elements which form the solid support of the invention comprises plastic beads.

8. A solid assay support according to claim 7 in which the beads are made of a polystyrene.

9. A solid assay support according to any preceding claim in which the elements which form the support are fused or sintered together to form a solid.

10. A solid assay support according to any preceding claim in which the elements forming the support have a diameter in the micrometer to millimetre range.

11. A solid assay support according to any preceding claim which is formed from a plastic scintillation material.

12. A solid assay support according to claim 11 in which the plastic material includes a fluorophore.

13. A solid assay support according to claim 12 in which the fluorophore is diphenyloxazole (PPO) or 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP).

14. A solid assay support according to claim 11, 12, or 13 in which the support will scintillate in the presence of a suitable radioactive material present in a sample applied to the support.

15. A solid assay support according to any preceding claim which is translucent.

16. A solid assay support according to claim 14 which is transparent.

17. An assay method comprising applying at least one sample to be analyzed to a surface of a support in accordance with any preceding claim and assaying the or each sample for the presence or absence of an analyte in the sample and/or for a quantifiable or qualitative effect.

18. An assay method according to claim 16 in which the sample is in liquid form.

19. A solid support for a chemical reaction, the solid support comprising a plurality of interconnected substantially spherical elements.

20. A support according to claim 16, for use as a support for DNA or polypeptide synthesis.

21. A cell growth support comprising a multiplicity of interconnected substantially

spherical elements arranged to provide a multiplicity of interstitial spaces therebetween in which cells can grow in, between and/or on the spherical elements.

22. A method of growing cells, the method comprising growing the cells on and/or in the cell growth support of claim 18.

23. A method of growing cells according to claim 19 in which the cells are grown in the interstitial spaces of the cell growth support.

24. The combination of one or more assay supports in accordance with any one of claims 1 to 16 with a multiwell plate or dish in which individual supports are placed in at least one well of the plate to reduce the volume of the well.

FIG. 1.

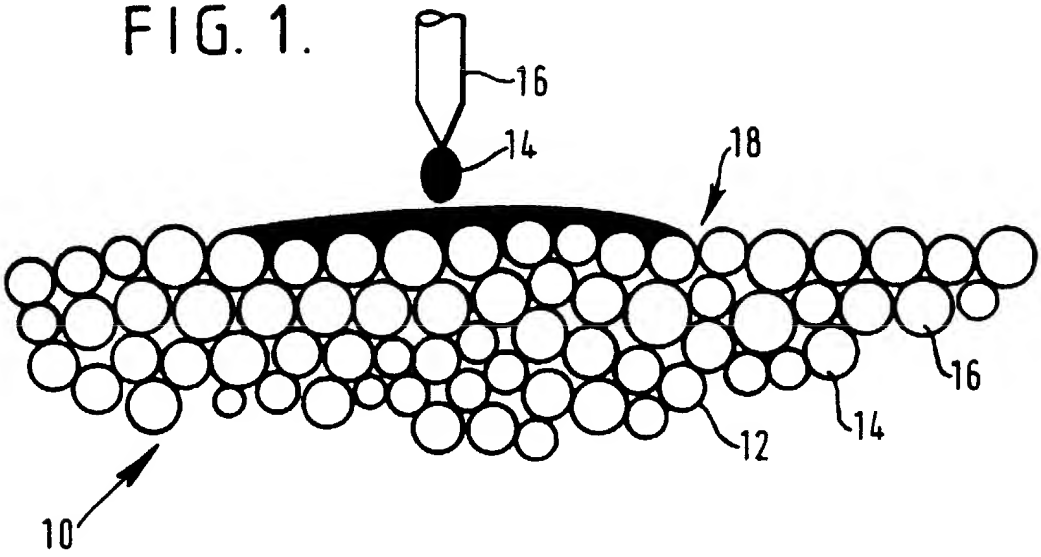
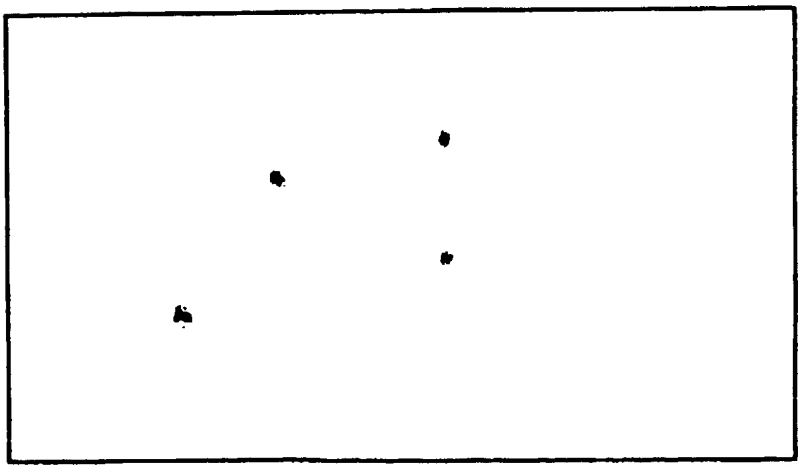


FIG. 2.



Binding competition test with SPLAT-tablets treated with hER-HBD extract, non-programmed yeast extract or plain buffer

Five concentrations of diethylstilbestrol, one concentration [3H]estradiol (=5 nM)

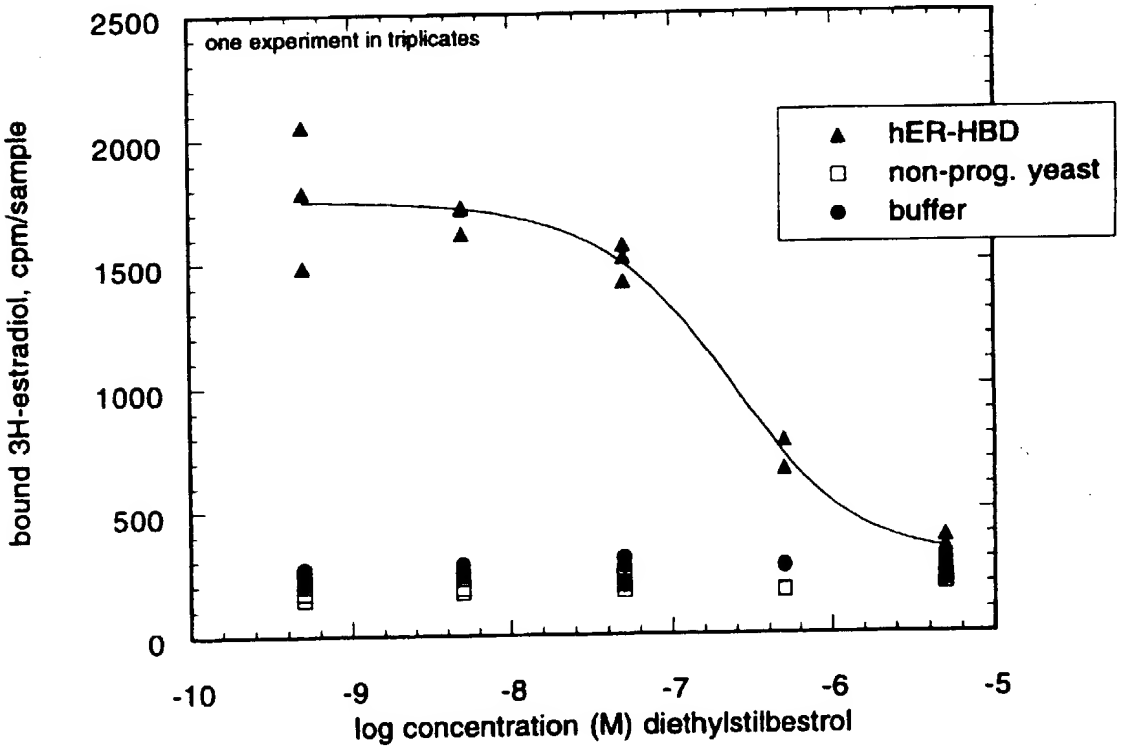


FIG. 3.

Simple binding kinetics; SPLAT-tablets with hER-HBD were treated with 30 µl 5 nM [3H]-estradiol. At steady-state a binding inhibitor was added at 3000x excess and compared to addition of vehicle alone

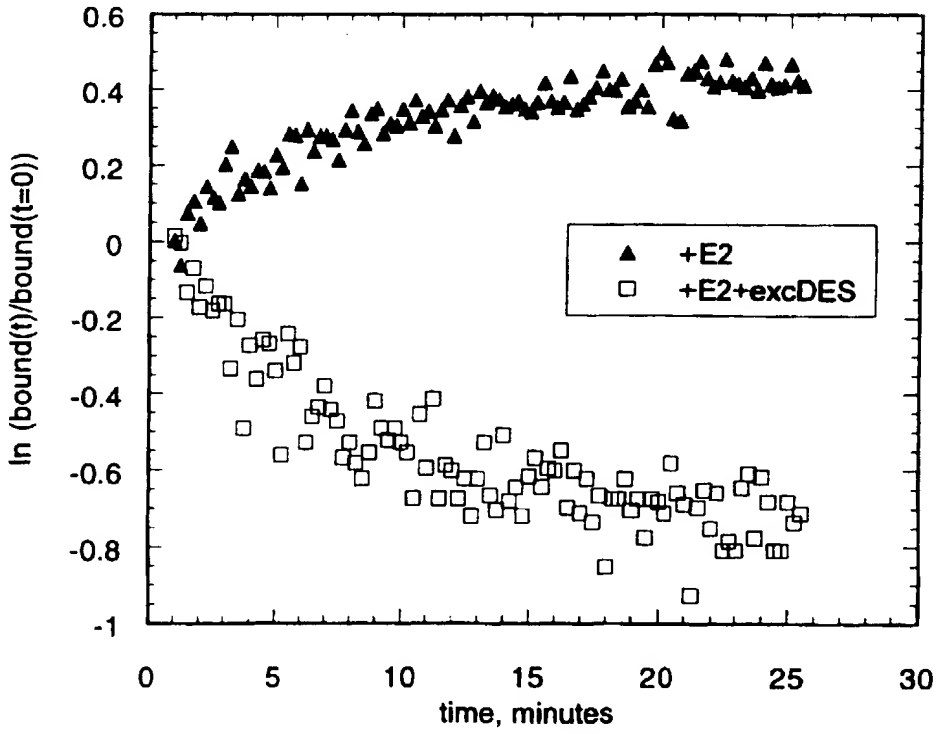


FIG. 4.